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Annotated Sequence Record

Complete nucleotide sequence of an isolate of coleus vein necrosis virus from verbena

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Introduction

Vegetatively propagated crops are particularly prone to virus infection, especially those that can be transmitted mechanically or by vectors that flourish in commercial production environments. Numerous viruses and viroids have been reported in *Verbena* × *hybrida* [17]. A *V.* × *hybrida* 'Taylor Town Red' plant exhibiting mottling, necrosis, and low vigor was received at the Oregon State University Plant Diagnostic Clinic from Illinois and tested positive for the presence of double-stranded RNA (dsRNA). The dsRNA was cloned and sequenced and was found to be nearly identical to a two-kilobase sequence of coleus vein necrosis virus (CVNV, Genbank DQ915963), a newly discovered carlavirus [14]. This communication reports the complete sequence of the verbena isolate of CVNV hereafter referred to as CVNV-V and provides data on biological properties of the virus including mechanical transfer to herbaceous indicators and identification of an insect vector.

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Virus material

Cuttings propagated from the original *V.* × *hybrida* 'Taylor Town Red' plant were used for purification of virus by the method of Martin and Bristow [12]. Virus purification yielded long, flexuous filamentous (639–877 × 13–14 nm, $n = 10$) and icosahedral virus-like particles, visible after negative staining with 2% ammonium molybdate. Members of the genus *Carlavirus* typically have filamentous particles of 610–700 × 12–15 nm [1]. DsRNA, purified using a method modified from that of Yoshikawa and Converse [22], was subjected to shotgun cloning [18] and sequenced on an ABI 3730XL DNA sequencer at Macrogen Inc. (Seoul, Korea). Using BlastX [2] at the National Center for Biological Information, 44 plasmids were found similar to carlavirus sequences, eight plasmids were similar to potyvirus sequences, and three plasmids were nearly identical to broad bean wilt virus-1. The sequence of the carlavirus was used to design primers for reverse transcription polymerase chain reaction (RT-PCR) to amplify the regions of the genome not obtained by shotgun cloning [19]. At least five sequence runs through each region of the genome were used to obtain a consensus genomic sequence (Genbank EF527260) using CAP3 [6].

For nucleic acids extraction and RT-PCR detection, the protocols described by Tzanetakis et al. [20] were employed. Primers F (5′-TAAGGGTGA CACTTCGGATG-3′) and R (5′-CGCAATGTGC TAACTTCACG-3′), which amplify a 300-base region of the coat protein (CP) gene, were used at a concentration of 1 μM each. Conditions used with a Stratagene Robocycler were: 3 min at 94 °C, followed by 40 cycles of 40 sec at 94 °C, 40 sec at 56 °C then 60 sec at 72 °C, followed by a final incubation at 72 °C for 4 min. The identity of the diagnostic PCR product was confirmed by sequencing amplicons from ten separate reactions.

Indicator plants were inoculated by rubbing a homogenate of virus-infected tissue at a ∼1:10 ratio (w/v) in 0.05 M phosphate buffer, pH 7.0, containing 2% polyvinyl pyrrolidone, onto carborundum-dusted leaves. The plants were tested by RT-PCR four weeks after inoculation. CVNV-V was detected in top leaves of 4/9 *Chenopodium quinoa* and in 8/8 plants of *Gomphrena globosa* but not in eight *Vigna unguiculata* subsp. *dekindtiana*, seven *Cucumis sativus*, or six *Brassica rapa* var. *rapa* plants.

Wingless green peach aphids (*Myzus persicae* Sulzer) were allowed to feed on virus-infected 'Taylor Town Red' plants for 24 h. Then, lots of 20 individuals were transferred to each of eight dsRNA-free *V.* × *hybrida* 'Twilight Blue with Eye', eight *C. quinoa* and four *G. globosa*. After a 24-h inoculation access feeding period, the plants were treated with Marathon®. Four weeks after insecticide treatment, shoot tips were evaluated for the presence of CVNV-V using RT-PCR. One of eight *V.* × *hybrida* 'Twilight Blue with Eye' and two of four *G. globosa* plants became infected with CVNV-V after the aphid transmissions. The results were verified by sequencing of the RT-PCR products. Aphid transmissions to *C. quinoa* were negative.

Sequence properties

The genomic sequence of CVNV-V is 8727 nucleotides long (47% GC content) exclusive of the poly(A) tail. The 2024 bases of the newly discovered carlavirus coleus vein necrosis virus [14] (Genbank DQ915963, hereafter referred to as CVNV-C) spanning from triple gene block (TGB) p2 to the poly(A) tail were 96% identical to the corresponding 2024 bases of CVNV-V, indicating that the two viruses belong to the same species. Open reading frames (ORFs) were identified using the NCBI ORF finder, and conserved domains were identified with the Conserved Domain Database at the NCBI using CD-Search [10]. The genome organization of CVNV-V was typical of that of other carlaviruses and contained six ORFs. Its 5′ untranslated region (UTR) is 73 and its 3′ UTR 101 nucleotides long excluding the poly(A) tail.

ORF1, predicted to yield a 222-kDa (1977 amino acids [aa]) protein, encodes the viral replicase, which contains methyltransferase, papain-like cysteine protease, helicase and polymerase motifs [7, 10] and is most closely related to poplar mosaic virus (PopMV) and garlic latent virus (GarLV) ORF 1 with 43 and 42% aa identity, respectively. ORFs 2, 3 and 4 form the TGB, encoding viral proteins involved in movement [15]. These ORFs have overlapping coding sequences in different reading frames and are most closely related to the PopMV orthologs. TGBp1 has a MW of 26 kDa (234 aa) and is 45% identical to the PopMV ortholog. The conserved helicase "P-loop" motifs I and II [21] were identified between residues 26–35 and 79–90, respectively. The putative role of the other two members of the TGB, TGBp2 and TGBp3, is to recruit TGBp1 to plasmodesmata and facilitate cell-to-cell movement [15]. TGBp2 MW of 12 kDa (108 aa) contains two transmembrane domains between residues 12–29 and 69–91 [9] which probably localize the protein in the endoplasmic reticulum and Golgi vesicles as in the case with TGBp2 orthologs [4, 15]. The protein has 92% nt and 94% aa identity to CVNV-C and 55% aa identity to PopMV TGBp2. TGBp3 is 7 kDa (68 aa) has a transmembrane domain between aa 10–29 [9] and shows 95% nt and 97% aa identity to CVNV-C and 44% aa identity to PopMV TGBp3.

The 34-kDa (315-aa) ORF5 was predicted to encode the CP of the virus, which is involved in genome protection and movement [3]. The CVNV-C and CVNV-V CP genes share nt and aa sequence identities of 96 and 95%, respectively. However, due to a base deletion, the deduced aa sequence of the CVNV-C ORF5 lacks 10 N-terminal aa and is thus smaller than that of CVNV-V. The CP of carnation latent virus (CarLV) is the closest relative to CVNV CP, with 64% aa identity. ORF6 was predicted to en-

code a 20-kDa (177-aa) nucleic-acid-binding protein (NABP), a putative suppressor of RNA silencing [11]. The CVNV-C and CVNV-V isolates share 98% nt and aa identity in the ORF 6 region. The garlic common latent virus (GCLV) NABP is the closest relative to CVNV ORF 6, with 44% aa identity.

Multiple sequence alignments and phylogenies were constructed with CLUSTALW [16] with its default parameters, after bootstrapping in 1000 pseudoreplicates. The phylograms of ORF1 (replicase) and ORF5 (CP) sequences of carlaviruses group CVNV with PopMV, GarLV, CarLV and GCLV (Fig. 1).

CVNV-C and CVNV-V are isolates of the same carlavirus species but have noticeable differences. CVNV-V is mechanically transmissible to *C. quinoa* and *G. globosa* and can be transmitted with the green peach aphid, unlike CVNV-C. These results may be due to the variability between isolates or the detection limitation of the techniques used in the CVNV-C experiments [14].

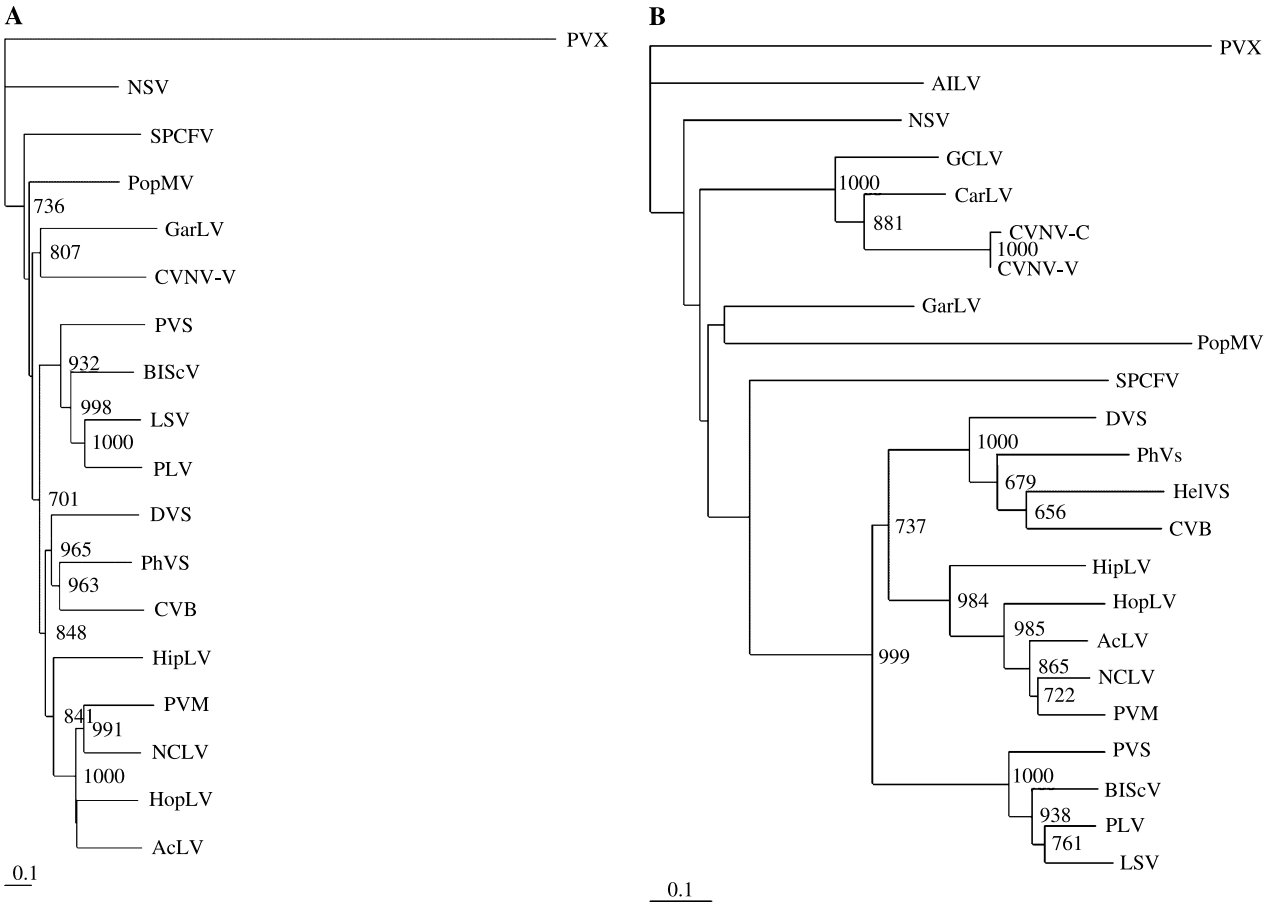

Fig. 1. Phylograms of the amino acid sequences of the replicase (**A**) and the coat protein (**B**) of carlaviruses. Abbreviations and GenBank accession numbers: aconitum latent virus (*AcLV*, NC_002795), alfalfa latent virus (*AlLV*, AY037925), blueberry scorch virus (*BlScV*, NC_003499), carnation latent virus (*CarLV*, X52627), chrysanthemum virus B (*CVB*, AJ585514), coleus vein necrosis virus-coleus (*CVNV-C*, DQ915963), coleus vein necrosis virus-verbena (*CVNV-V*, EF527260), daphne virus S (*DVS*, NC_008020), garlic common latent virus (*GCLV*, DQ52009), garlic latent virus (*GarLV*, NC_003557), Helenium virus S (*HelVS*, D10454), hop latent virus (*HopLV*, NC_002552), hippeastrum latent virus (*HipLV*, DQ098905), lily symptomless virus (*LSV*, NC_005138), narcissus common latent virus (*NCLV*, NC_008266), narcissus symptomless virus (*NSV*, NC_008552), passiflora latent virus (*PLV*, NC_008292), phlox virus S (*PhVS*, NC_009383), poplar mosaic virus (*PopMV*, NC_005343), potato virus M (*PVM*, NC_001361), potato virus S (*PVS*, NC_007289), potato virus X (*PVX*, NC_001455), sweet potato chlorotic fleck virus (*SPCFV*, NC_006550). The numerical values of the nodes with bootstrap values of less than 60% (600) are not shown, as they are not considered significant. PVX ORF1 and ORF5 are used as outgroup sequences. The bars represent 0.1 amino acid changes per site

Many of the newly described viruses of verbena have been found in symptomatic plants that contained multiple viruses [5, 8, 13]. These plants likely became infected via multiple inoculation events either by mechanical or vector transmission. This is the first report of CVNV in verbena, and the data presented in this communication contributes to a better understanding of the nature of plant viruses in the floriculture industry and may lead to developing better management strategies. Efforts to characterize the novel potyvirus identified in the symptomatic 'Taylor Town Red' are underway. Information on the other viruses present in the diseased 'Taylor Town Red' and re-creation of the mixed infections will be necessary to determine if single infections with CVNV-V or mixed infections are required for symptom development in this cultivar.

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